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ZIP9 but not the androgen receptor mediates testosterone-induced migratory activity of metastatic prostate cancer cells

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ABSTRACT

LNCaP cells are derived from a metastatic lesion of human prostate adenocarcinoma. They express the classical androgen receptor (AR) and ZIP9, a Zn^{2+} transporter that also binds testosterone and mediates signaling by interacting with G-proteins.

Our results show that LNCaP cells respond to testosterone by mobilizing their migratory machinery. Their exposure to testosterone triggers the formation of lamellipodia, reorganization of the actin cytoskeleton, phosphorylation of focal adhesion kinase (FAK) at Tyr925 and of paxillin at Tyr118, expression of matrix metalloproteinase 2 (MMP-2), and cell migration.

Silencing ZIP9 expression by means of siRNA does not affect the responsiveness of the classical AR to testosterone; however, it prevents all of the testosterone effects described above: formation of lamellipodia cannot be induced, stimulation of FAK or paxillin phosphorylation or MMP-2 expression is prevented, and cell migration does not take place in the absence of ZIP9.

The data presented show that testosterone/ZIP9 interactions might have not only physiological but also pathophysiological relevance. The fact that the migratory machinery of a metastatic prostate cancer cell line is activated exclusively through testosterone/ZIP9 and not through testosterone/AR interactions suggests that targeting specific inhibition of testosterone/ZIP9-mediated events might help in developing new therapeutic strategies against androgen-induced progression of prostate cancer.

1. Introduction

Androgens like testosterone (T) and its primary metabolite in the prostate, dihydrotestosterone (DHT), direct not only the normal development and function of the prostate gland [1] but also modulate pathological events such as age-related prostate carcinoma and benign prostatic hypertrophy [2] and regulate prostate tumor progression and metastasis [3]. Since 80–90% of all prostate cancers depend on androgens, their treatment focuses mainly on the reduction of serum androgens [4] and on the suppression of the androgen receptor (AR) axis [5–7] either through surgical or hormonal castration (androgen ablation therapy). Anti-androgens like bicalutamide (Casodex) or flutamide that bind to the classical AR are applied in order to further suppress androgenic activities [4].

Despite such therapy, however, prostate cancer can recur and resume growth even though plasma androgen levels are low and AR-mediated effects are kept at a minimum by anti-androgens [8]. Several of these so-called castration-resistant prostate cancers are metastatic and do not express the classical androgene receptor (AR), an intracellular transcription factor that becomes activated by testosterone or DHT [9,10]. Thus, it is justified to assume that prostate cancer therapies aimed solely at the prevention of nuclear translocation of the classical AR and its associated genomic effects [4,11] might not be sufficient for the complete abrogation of androgen signaling. Identification of alternative pathways of androgen-induced signaling might help to pinpoint reasons for resistance of various prostate tumors to current anti-androgens and to develop new treatment methods that are aimed at abrogation of all androgen-induced signaling, both classical and non-classical.

Recent investigations demonstrate that the zinc transporter ZIP9 from the family of the ZRT, IRT-like proteins (ZRT = zinc-regulated transporter; IRT = iron-regulated transporter) is a membrane-bound

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Table 1
Antibodies used and their providers.

Antibody	Provider	Article number	Application in the current studies
Anti-paxillin (phospho-Tyr 118)	**Cell Signaling**, Frankfurt Main, Germany	2541	Western blotting, immunofluorescence
Anti-focal adhesion kinase (phospho-Tyr 925)	**Cell Signaling**, Frankfurt Main, Germany	3284	Western blotting, immunofluorescence
Anti-focal adhesion kinase (total)	**Cell Signaling**, Frankfurt Main, Germany	3285T	Western blotting
Anti-paxillin (total)	**Bio-Techne GmbH**, Halle, Germany	4259	Western blotting
Anti-MMP-2	**Millipore**, Darmstadt, Germany	MAB3308	Immunofluorescence
Anti-ZIP9	**Novus Biologicals**, Wiesbaden-Nordenstadt, Germany	NBP1-83760	Western blotting, immunofluorescence
Anti-vinculin	**Sigma-Aldrich**, Taufkirchen, Germany	V9264	Western blotting
Anti-actin	**Cell Signaling**, Frankfurt Main, Germany	4967	Western blotting
Anti-actin	**Abcam**, Cambridge, United Kingdom	Ab8226	Immunofluorescence
Anti-AR	**Santa Cruz Biotechnology**, Heidelberg, Germany	sc-13062	Immunofluorescence
Anti-PCNA	**Cell Signaling**, Frankfurt Main, Germany	2586	Western blotting

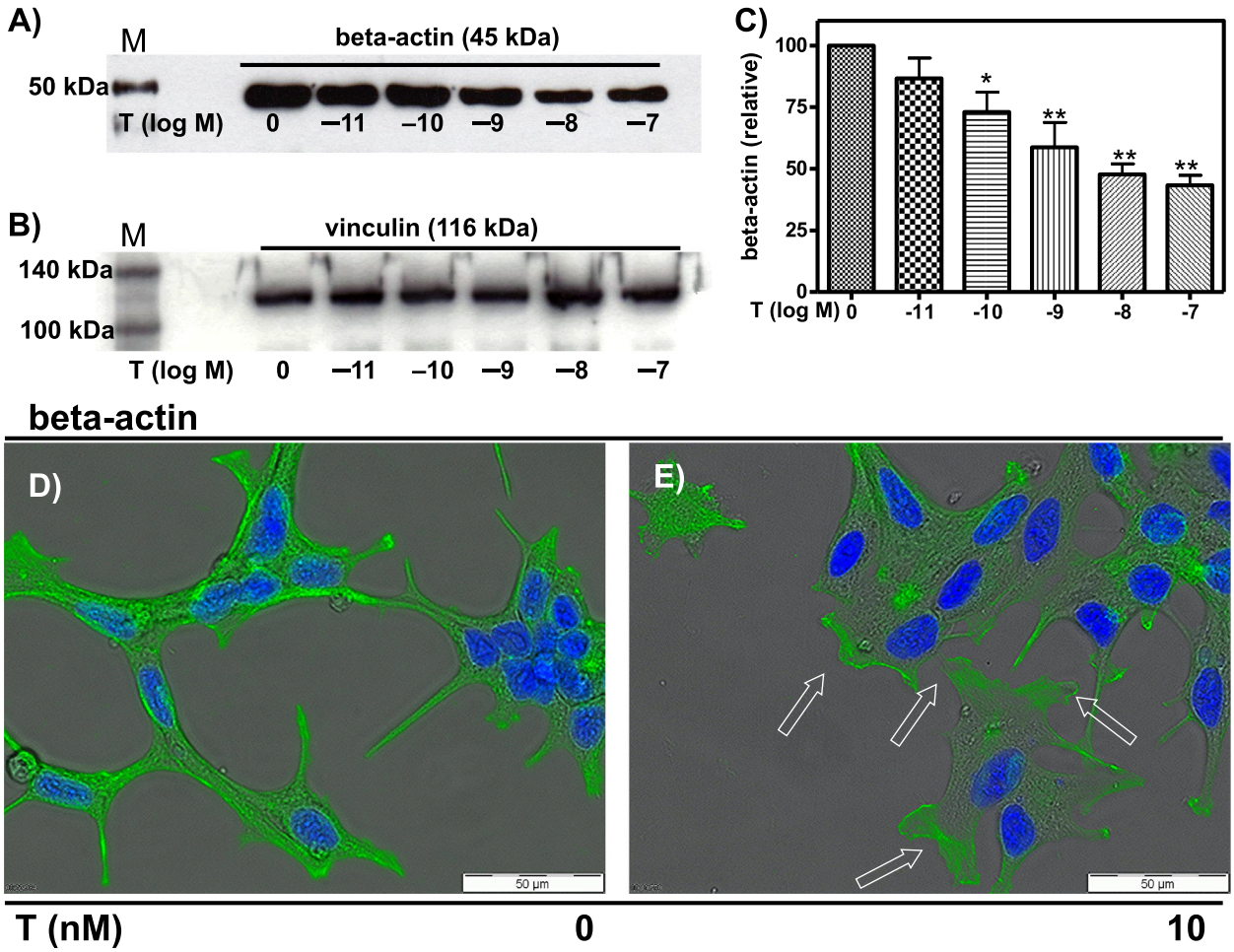

Fig. 1. Effects of testosterone on actin expression and lamellipodia formation. (A) Western blot demonstrating reduction of beta actin levels in LNCaP cells as a function of the concentration of testosterone [T]. (B) Expression of vinculin is not affected by T. (C) Statistical analysis of beta actin signals from 3 independent Western blots (means ± SEM; $*p \leq 0.05$; $**p < 0.01$). (D) Detection of beta actin by immunofluorescence in the absence of T (green fluorescence). Nuclei are stained blue with DAPI. (E) Exposure to T triggers reshaping of the cells characterized by widened cell bodies and formation of lamellipodia (arrows). The strongest actin signals are seen within lamellipodia. The photomicrographs consist of merged bright field and fluorescence images.

receptor for T that mediates T signaling events through interactions with G-proteins [12–14], which might be of physiological relevance. Thus, in spermatogenic cells ZIP9 mediates the so-called non-classical signaling pathway of testosterone, characterized through activation of Erk1/2, CREB and ATF-1 [15] and in Sertoli cells, by activating the same pathway, it stimulates claudin expression and formation of tight junctions [16]. ZIP9 can also serve as a pharmacological target of anti-androgens, as ZIP9-mediated effects of T can all be inhibited by bicalutamide [17], a widely used drug for treatment of androgen-dependent malignancies [4]. A possible direct involvement of ZIP9 in the progression of these malignancies, however, has not yet been addressed.

LNCaP cells, derived from a metastatic lesion of human prostate adenocarcinoma [18], are androgen sensitive and have maintained their metastatic behavior. Earlier investigations revealed that exposure of these cells to testosterone-BSA triggers phosphorylation of focal